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Assessment of the urban trees health status on the base of nutrient and pigment content in their leaves

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ABSTRACT

Town settlements have different load level by emissions originated mostly from transport, industry and heating system. Their environmental and climate conditions are more or less changed that effect to growth, physiology and vigor of woody plants at the city public vegetation areas. Our study on determining the impact of urban environment on the tree health status was focused on the quantities of nutrients and main components of the pigment complex – chlorophyll *a*, chlorophyll *b* and carotenoids. Leaves of *Acer platanoides* L., *Aesculus hippocastanum* L. and *Betula pendula* Roth. were sampled from urban areas with different type of anthropogenic pressure in the town of Plovdiv (Bulgaria). Concentrations of the elements Ca, K, Mg, N, Na, P, and S were analyzed by ICP-MS. Health condition of trees in the city parks and suburban areas was acceptable, but in the central part and close to the industrial area it was non-satisfactory. This preliminary research pointed ecophysiological tools as useful to develop new criteria for sustainable urban arboriculture, including species selection (based on stress tolerance criteria), nursery hardening and preconditioning, and care after planting.

Key words: *Acer platanoides*, *Aesculus hippocastanum*, *Betula pendula*, health status, urban vegetation, biomonitoring

Introduction

Town settlements are characterized with comparably high emission load and synanthropically changed climate, which creates worsened living conditions for plants, animals and humans (Takuchev, 2011). Urban vegetation has important ecological, environmental improving, aesthetic and cultural functions in settlement conditions (Feriancová, 2003; Supuka et al., 2004, 2005; Kaliszuk & Szulczewska, 2006; Rehackova & Pauditsova, 2006). Tree status in the city can be influenced by many factors, such as application of de-icing materials during winter months, soil structure and chemical properties, microclimate, supply with biogenous elements, soil and air pollution, insect damages, etc. All of the above mentioned features alter tree physiology, productivity and vigor. The health of trees in the cities of northern Europe and North America is poor, resulting in massive die-offs - in the cities of Western Europe, over 700 000 trees die annually (Fluckinger & Braun, 1981). This situation was found in urban vegetation areas at woody plants e.g. *Acer pseudoplatanus, Aesculus hippocastanum, Betula pendula, Corylus avellana, Tilia cordata, Pinus sylvestris* (Hrdlicka & Kula, 1988; Somsak et al., 2000; Kontrišová & Kontriš, 2001; Supuka, 2001; Tomasevic et al., 2005; Mitrovic et al., 2006).

This study was conducted on three deciduous tree species which are frequently used for landscaping green areas in the cities and especially along transportation routes in many European cities – Norway maple (*Acer platanoides* L.), Horse chestnut (*Aesculus hippocastanum* L.) and Silver birch (*Betula pendula* Roth.). *A. platanoides* is considered as tolerant plant to industrial contamination and it is recommended for construction of forest belts around the point sources of air pollutants (Piszak et al., 2002; Dineva, 2006). There are conflicting reports regarding *A. hippocastanum* tolerance to present-day urban conditions. Some European scientists define this species as highly sensitive (Suchara, 1982), whereas US references consider

RESEARCH ARTICLE

this species to be resistant to salinity (Johnson & Sucoff, 1999). *B. pendula* is proved to be very useful as a biomonitor of trace element contamination because of its resistance to industrial type of pollution (Ivanova & Velikova, 1990; Kozlov et al., 1995).

The nutrient and pigment concentrations in the leaves could be considered as functional and physiological criterion both for the health and vigor of plants, and for the environmental conditions (Hrdlicka & Kula, 2001; Cekstere et al., 2008). The plant leaf test represents not only the effects of soil nutrient status and the degree of pollution, but also all the factors controlling plant growth (Cekstere et al., 2005). The visual symptoms of heavy metal damages on trees as well as the imbalance in the system of plant mineral nutrition does not always appear in the leaves in the begging, although at the same time tree growth and development could be seriously inhibited.

In order to assess the impact of urban environment on the health status of selected urban trees, we explored the macro element concentrations and the content of photosynthetic pigments as key limiting factors for tree biology. This preliminary research was based on the hypothesis that plant leaf analysis could detect early toxicities or hidden deficiencies and this kind of information might be of diagnostic assistance for the planning and management of the urban green system.

Materials and Methods

Study area and sampling sites

The research was conducted in Plovdiv (N42°9', E24°45'), which is one of the biggest and most densely populated towns in Bulgaria – 338 000 inhabitants on 102 m^2 area (Census, 2011, NSI). It is characterized by specific topography and meteorological conditions – six syenite hills inside the city, wide network of busy streets and train tracks, many tall buildings, high percentage of days with fog and lack of wind, leading to retention of contaminants.

For the purposes of our study, five sites were selected on the basis of typology of urban green areas and the type of anthropogenic impact. The selection of sites was based on the presence of tree species with similar age (40-60 years old) and on the identification of common features in trees like size, and canopy density.

North site (N42º10'32.3", E24º45'53.4") was chosen in peri-urban settings, close to an industrial sector (North industrial zone in the town of Plovdiv) with fossil fuel power plant, cosmetic production, glass producer and other small point sources of contaminants. Examined trees were growing in a large green area, located to a distance of about 1 km to the East of the industrial zone.

East site (N42º08'24.3", E24º47'06.2") was situated in a housing estate "Trakiya", and was characterized with high traffic and moderate household's pressure. Selected trees were growing in small green patches, 5-10 m away from tall buildings.

South-East site (N42º07'42.2", E24º47'40.5") was also chosen in "Trakiya", but the trees were located in a small green patches near (50-100 m) to a Railway station.

Central site (N42º08'22.8", E24º44'24.1") was situated in the real center of the town of Plovdiv, along one of the major streets (Ruski Blvd.) of intensive daily traffic. The examined trees were growing in a big city park (Natural monument "Bunardzhik"), on the East slope of Bunardzhik Hill, 10-20 m away from the boulevard.

West site (N42º07'54.4", E24º42'18.0") was chosen in a housing estate "Smirnenski", subjected to a moderate anthropogenic impact. Examined trees were located in small green patches around the buildings (5-10 m distance) and similar to the other sites.

Leaves for analysis were sampled in the end of June and in the middle of September 2010. Leaf samples were taken with telescopic scissors from the outer part of the tree crown, at the 2.5–3 m height in all directions. At least five trees per species in each site were studied. Usually 80-100 fully expanded leaves of each one tree were collected and a composite sample was prepared for analyses.

Plant analysis

Pigment analysis followed Schlyk (1965) and was made as quickly as possible after sampling. Spectrophotometric reading was performed after extraction with 90% acetone at the Faculty of Biology, Plovdiv University. Concentrations of chlorophyll *a*, chlorophyll *b*, total chlorophyll and carotenoids were calculated for each sample and presented in mg g^{-1} fresh weight (Petrova, 2011).

The leaf samples were not washed after collection. In determining the macro elements, no washing is applied because some of them (like K and Na) could be washed away, thus distorting the results (Dmuchowski et al., 2011). Plant material for chemical analyses was air dried for two weeks, ground to a powder and homogenized. The content of Ca, K, Mg, Na, P and S was determined by inductively coupled plasma mass spectrometry (ICP-MS) using

RESEARCH ARTICLE

instrument Agilent 7700 ICP-MS (2009), DF 1000 (Petrova, 2011). All samples, blanks and standards were spiked with international standards. Calibration standards Multy VI (MERCK) were freshly prepared from 1 to 1000 ppb in 0.05% HNO_3 (p.a.). Quality control was performed using the standard reference plant material (NCS DC73348).

Data processing

For all statistical analyses the STATISTICA 7.0 package (StatSoft, 2004) was used. The concentrations were expressed as arithmetic means and standard deviation, and the differences were considered significant when $p<0.05$ based on the one-way analysis of variance (ANOVA). Relationships between the contents of individual elements in collected leaf samples were tested using Spearman rank correlation coefficients. Multivariate evaluation of the similarity in nutrient and pigment concentrations in the examined deciduous trees from five different urban areas was conducted using cluster analysis. Next the data were processed with Principal components and classification analysis ($p<0.05$).

Results

Nutrients

The element concentrations in leaf samples of *A. platanoides*, *A. hippocastanum* and *B. pendula* from June 2010 were presented in Table 1. Some differences were found between plant species and also between studied sites. The descending order of nutrients in maple leaf samples was as follows: K > Ca > Mg > P > N > S > Na, $p<0.05$. In chestnut leaves it was very similar: K > Ca > Mg > P > N > S > Na, $p<0.05$. Only in birch leaves another tendency was found: Ca > K > Mg > S > P > Na , $p<0.05$.

Highest levels of the elements Ca, K and N were found in the leaves of *A. platanoides*, followed by *A. hippocastanum*, where maximal values of Mg and Na were also measured. In *B. pendula* leaf samples only S content was higher than in the other two species, probably due to its bioaccumulation ability.

Higher content of N and K and lower content of Ca, Na, P and S was found in leaf samples from West area. Maximal concentrations of Ca, P and S were measured in Central area, followed by South-East and East area. In North, Central and East area quite elevated concentrations of Na were determined.

Relationships between macro elements were studied in the collected foliage samples. Cluster analysis divided them in two major groups. Strong proximity and similar attitude was found between K, Na and P. The second cluster consisted of Ca, S and Mg. Inter element correlation coefficients were calculated and significant differences in nutrient coexistence were observed depending on the tree species and the sampling site. Positive relationships in *B. pendula* leaf samples were found between K-Ca, Na-P, P-S; in *A. hippocastanum* – positive correlation existed between K-P, Ca-P, Ca-S, Mg-N, and negative between Mg-P and Ca-N. In *A.platanoides* leaves, two positive (Na-P, S-N) and three negative relationships (Ca-K, Na-N and Na-S) were established.

The results from the PCA analysis were shown on Figure 1. Sulfur and magnesium concentration were found to be much more influenced by plant species than to tree location. Sodium content was dependent almost only of the sampling site. The observed level of elements Ca, P and to K in foliage samples was mainly due to the sampling site location but also of the tree species.

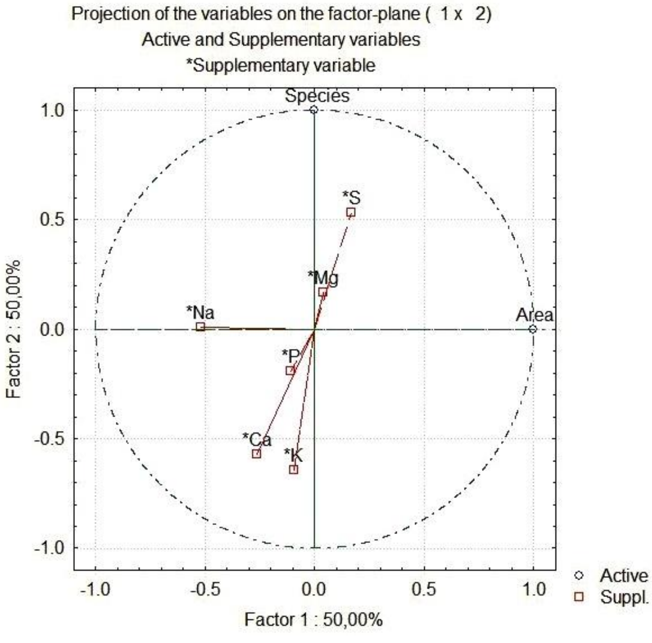

Figure 1. *Relationships between plant species, sampling area and nutrient content: axis x represented the significance of the area location, axis y represented the significance of the plant species, p<0.05.*

RESEARCH ARTICLE

Table 1. *Mean values and standard deviations of the nutrient content in tree leaves (June 2010) according to sites, expressed in mg kg^{-1} dry weight.*

Element	Tree species	North area	East area	South-East area	Central area	West area
Ca	*A. platanoides*	14832±371	16488±379	18336±532	18435±295	13727±371
	A. hippocastanum	13341±494	14319±358	13483±337	15000±150	13562±448
	B. pendula	15049±120	16535±265	12876±116	11513±81	11391±171
K	*A. platanoides*	18471±499	18513±463	18653±522	12397±186	20616±577
	A. hippocastanum	17434±488	15889±461	13719±329	20900±418	19036±495
	B. pendula	16217±81	12525±150	10012±120	11003±66	10058±141
Mg	*A. platanoides*	2709±62	2144±41	3763±45	2340±14	2631±87
	A. hippocastanum	3565±61	2611±44	6116±49	3900±62	3451±14
	B. pendula	3050±15	2570±41	4500±86	2620±42	2900±35
N	*A. platanoides*	2510	2010	2600	2040	2380
	A. hippocastanum	1440	1970	1800	1830	2250
	B. pendula	n.a.	n.a.	n.a.	n.a.	n.a.
Na	*A. platanoides*	74±1.4	21±0.7	17±0.6	31±0.4	13±0.5
	A. hippocastanum	156±2.2	171±2.4	30±0.7	32.2±0.4	14±0.3
	B. pendula	30.2±0.3	29.8±0.2	27±0.7	47.3±0.7	25.9±0.3
P	*A. platanoides*	3160±63	1610±37	1758±33	2742±36	2452±29
	A. hippocastanum	2615±52	2922±108	1139±15	3100±22	2201±42
	B. pendula	2079±21	1919±15	2271±9	2529±20	1577±6
S	*A. platanoides*	1940±126	2109±89	2108±82	2207±135	2401±108
	A. hippocastanum	1140±112	1694±73	1764±12	2300±46	1683±62
	B. pendula	2920±152	2861±160	3021±121	2835±74	2506±90

Data from measured trace elements concentrations in collected leaf samples from the three studied plant species were processed with cluster analyses in order to find some differences between selected sampling sites (Figure 2). Strong proximity was demonstrated by North and West area, and also between East, Central and South-East area.

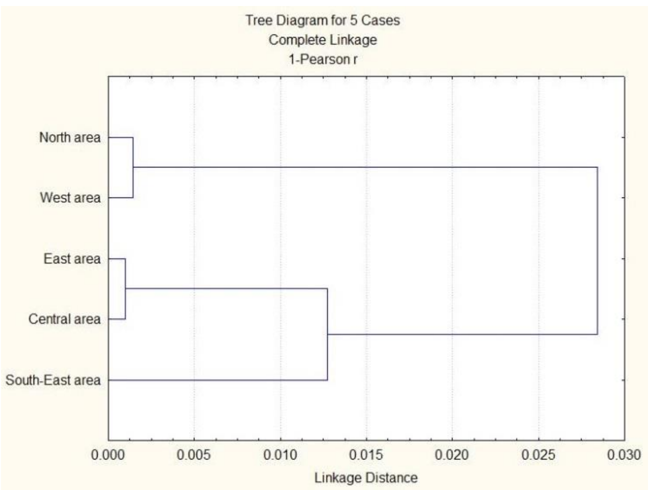

Figure2. *Cluster analysis of the studied urban areas on the base of nutrient content in the sampled tree leaves, p<0.05.*

Pigments

The pigment content was significantly different between plant species, sampling sites and seasons (Figure 3). Higher chlorophyll concentrations were determined for *A. platanoides*, followed by *A. hippocastanum* and *B. pendula* in both studied periods. Carotenoids content was quite elevated in *A. hippocastanum* leaves than in the other two plant species.

As a rule, the leaf samples showed decrease in pigment values in the autumn. Only few exceptions were observed, as follows: chlorophyll content in *A. platanoides* leaves from West area, chlorophyll and carotenoid content in *A. hippocastanum* and *B. pendula* samples from North area, carotenoid concentration in all three studied species from Central area. Ratio between chlorophyll and carotenoid concentrations also demonstrated decreasing tendency from June to September. Exception was noticed in West sampling area where we obtained from 10% to 35% higher values of this parameter in all autumn leaf samples.

In June, the leaves from *A. platanoides* showed highest chlorophyll content (3.698±0.37 mg g^{-1}) and lowest carotenoid content (1.292±0.002 mg g^{-1}) in North area, and

RESEARCH ARTICLE

respectively minimal chlorophyll (3.106±0.42 mg g^{-1}) and maximal carotenoids (1.546±0.14 mg g^{-1}) in West area. In September, chlorophyll concentration varied from 2.279±0.11 mg g^{-1} (East area) to 3.376±0.12 mg g^{-1} (West area) and carotenoids varied from 1.156±0.002 mg g^{-1} (North area) to 1.656±0.05 mg g^{-1} (Central area).

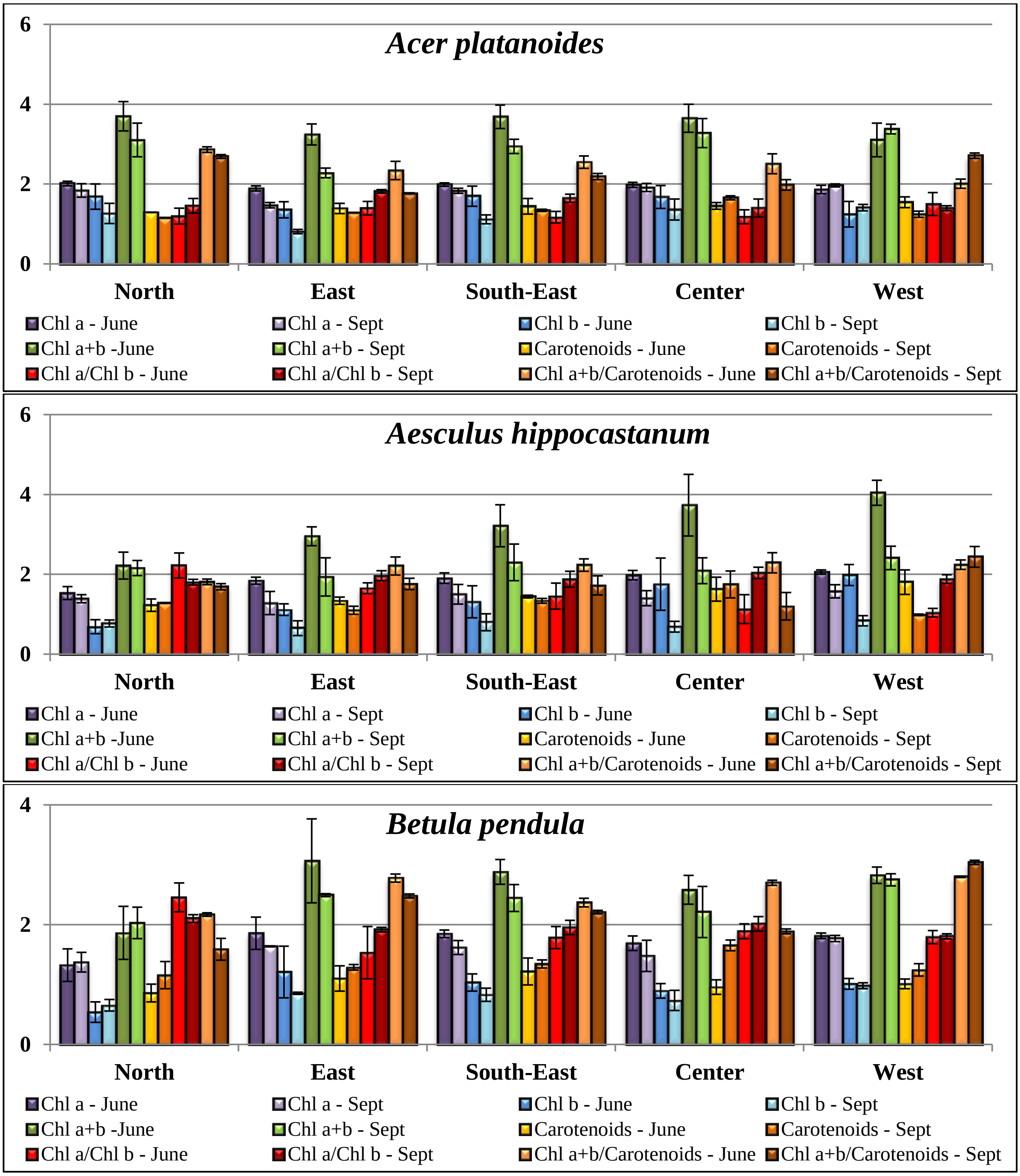

Figure 3. *Mean values and standard deviations of the pigments content in tree leaves according to sites, expressed in mg kg^{-1} fresh weight.*

RESEARCH ARTICLE

In *A. hippocastanum* leaves, spring minimum of both chlorophyll (2.219±0.34 mg g^{-1}) and carotenoid (1.227±0.16 mg g^{-1}) concentrations was measured in North area, while both maximums were found in samples from West area - 4.042±0.31 mg g^{-1} and 1.806±0.31 mg g^{-1}, respectively. Autumn chlorophyll values were lowest in East area (1.935±0.48 mg g^{-1}) and highest in West area (2.412±0.29 mg g^{-1}), but carotenoids concentration was minimal in West (0.986±0.02 mg g^{-1}) and maximal in Central (1.745±0.34 mg g^{-1}) area.

For the *B. pendula* leaves, lowest chlorophyll (1.863±0.44 mg g^{-1}) and carotenoid (0.859±0.15 mg g^{-1}) level in the spring was measured in North area. Maximal values for green pigments (3.067±0.7 mg g^{-1}) were obtained from East area and for orange pigments – for South-East area (1.218±0.23 mg g^{-1}). In the autumn, we observed minimal chlorophyll content in North area (2.028±0.26 mg g^{-1}) while maximum was found in West area (2.75±0.1 mg g^{-1}). On the contrary, maximum carotenoids (1.277±0.23 mg g^{-1}) were determined in birch leaves from North area and minimum – in those from West area (0.904±0.1 mg g^{-1}).

It is widely known that leaf chlorophyll content is an important parameter for testing plant status: it can be used as an index of the photosynthetic potential as well as of the plant productivity (Carter, 1998). In addition, chlorophyll gives an indirect estimation of the nutrient status (Filella et al., 1995) and together with carotenoids is closely related to various types of plant stresses and senescence (Gitelson & Merzlyak, 1994). For this reason we aimed to test the relationships between studied plant species, selected sampling sites, sampling period and data obtained. Statistical evaluation showed that pigment content was quite species specific, but the other two parameters – ratio chlorophyll *a/b* and especially the ratio chlorophyll *a+b*/carotenoids, were strongly dependent from the sampling area and sampling season (Figure 4). On the base of these two ratios, cluster analysis was performed. Central, East and South-East areas formed one major cluster like in previous analysis on the base of nutrient concentrations. But in this case, West area demonstrated closer proximity to the mentioned areas than to the North one (Figure 5).

Discussion

The forests and plantations in the green system of the town of Plovdiv are a typical example of ecosystems that influence and are influenced by the urban environment and the industrial activity. Increasing or decreasing of element level in plant organs was depended on either abundance of deficiency of those elements in soil or in air. Town conditions are specific, where increased amount were found in air and soil as mark of environmental load. This situation was related to the element accumulation increasing in leaves, disturbing the phenology and physiology cycles and worsened healthy conditions of plants.

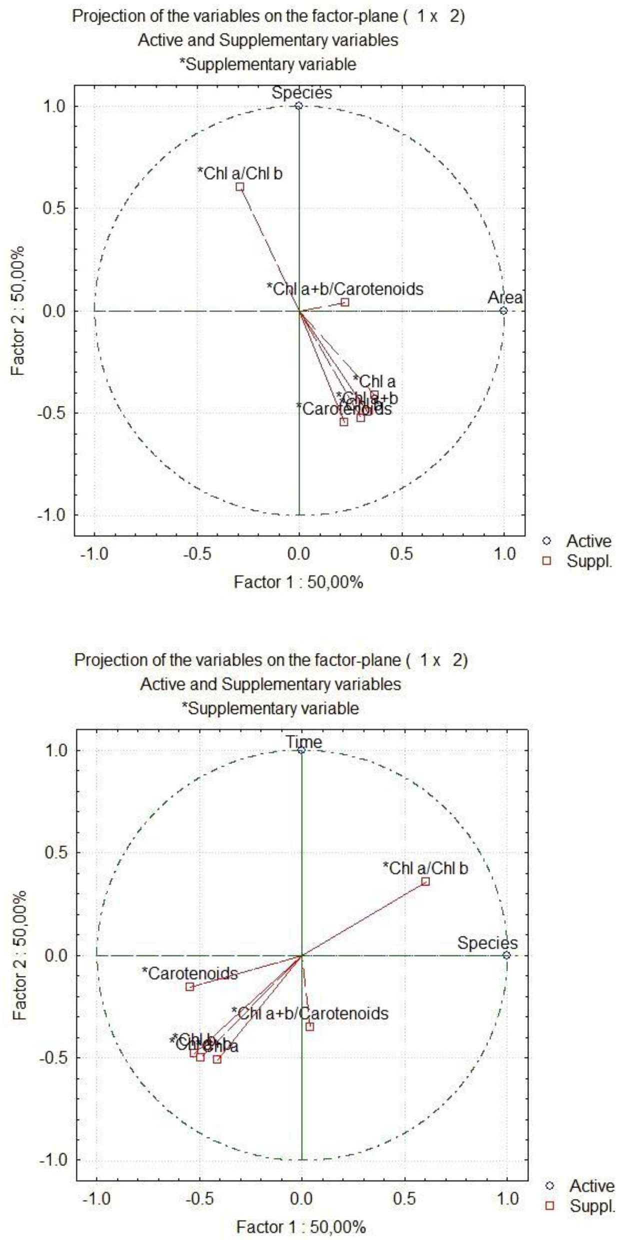

Figure 4. *Relationship between plant species, sampling area, sampling season and pigment content in the tree leaves, p<0.05.*

RESEARCH ARTICLE

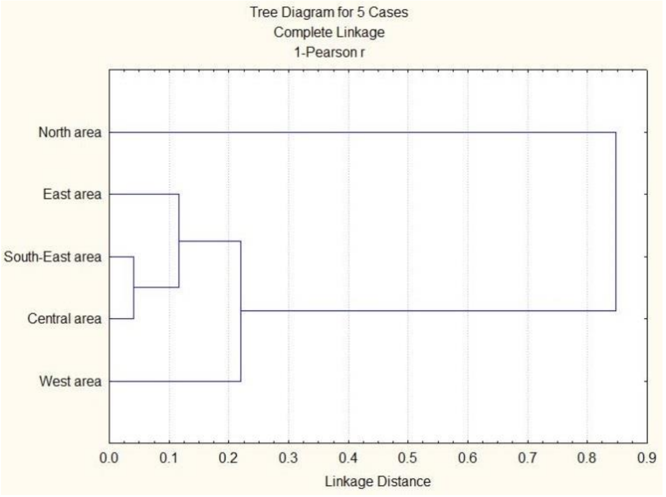

Figure 5. *Cluster analysis of the urban areas on the base of pigment content in sampled tree leaves, p<0.05.*

Examined trees of *A. platanoides* showed increased K and S concentrations in West area according to the optimal values for this species given by Kopinga & van den Burg (1995). Data from East and West area about leaf content of Mg (in regard to K) tended to possible deficiency. Some K deficiency (in regard to Ca) also could be supposed, especially in Central and South-East area (Kopinga & van den Burg, 1995). Na and P content in leaves from North area were greater than in the others but all stayed in normal range. The proportion of nutrition elements to N were also in the optimum. Our results about Na content were quite lower in comparison with data from Sofia given by Gateva (2004).

The results obtained on the nutritional status of studied *A. hippocastanum* trees in Plovdiv showed decreased levels of P and K and a possible deficiency of K with regard to Ca and of P with regard to N in South-East area (Kopinga & van den Burg, 1995). Excess of K and P, combined with elevated concentrations of S and Ca, we observed in Central area. Natural limit content of Mg was considered between 2.0-4.0 mg g^{-1} (Bublinec, 1990) and only in South-East area we detected an excess in all three studied species. These values were close to data from Sofia reported by Gateva (2004). Probably the studied trees in West area had suffer from sodium deficiency while in the leaves from both North and East area was detected an excess of sodium - 56-70% up to the normal content given by Bublinec (1990).

Our results showed that concentrations of Na and Mg in all sampled leaves of *B. pendula* were in normal range, but S level was elevated in South-East and North area (Kopinga & van den Burg, 1995). Data concerning average Mg concentration in our leaf samples were significantly higher to the urban site of Nitra town and were close to the control one (Supuka et al., 2008). Phosphorus values from West and East area also were critical for *B. pendula* health status. As a normal content of Ca for this species were considered 3.0-15.0 mg g^{-1} (Bublinec, 1990) and according this criterion the monitored trees from North and East area in Plovdiv had excess of this element. The average content of Ca, found by us, was close to the values reported from urban site in Nitra town, Slovakia (with heavy motor traffic) but quite lower when compared with the control forest site (Supuka et al., 2008). Highest Ca content in the other two species we found in Central area which could be explained with the presence of this element in dust pollution and the bigger surface area of the *A. hippocastanum* and *A. platanoides* leaves, thus bigger trapping efficiency. Data about K content were above the upper limit, especially in North area (65%), but some deficiency with regard to Ca content could be supposed (Kopinga & van den Burg, 1995). Our results were significantly elevated when comparing to data from forest sites reported by other authors (Reimann et al., 2007; Supuka et al., 2008). The average potassium content in all studied plant species was from 1.5 to 2 times greater in Plovdiv than in the same species leaves from Sofia (Gateva, 2004).

Our studies on determining the impact of urban environment on the tree health status were also focused on the quantities of the main components of the pigment complex – chlorophyll *a*, chlorophyll *b* and carotenoids. Their amounts normally decrease from June to September and together with leaf ageing the decomposition of chlorophyll *a* was faster than chlorophyll *b*. Our results showed a complex situation in this respect when a comparison was made between selected sampling sites, and chlorophyll *b* was found as more susceptible to pollution.

The changes in the chl *a/b* ratio and chl/carotenoid ratio (which had the advantage to be dimensionless parameters) could be used as more informative indicators than pigment concentration in ecological investigations. Typically, decreases in chl *a/b* ratios were observed during senescence. In this study, we found an increase in the ratio of chl *a/b* during autumn in 85% of all leaf samples. Probably, the major reason for that was the stronger decrease in chl *b* content, than in chl *a*. The decrease of chlorophyll content may be due to an increase of chlorophyll degradation or to a decrease of chlorophyll synthesis. During the process of chlorophyll degradation, chl *b* is converted in chl *a* (Fang et al., 1998) and this may explain the increase of the ratio chl a/b together with the depression of chlorophyll content.

Sillanpää et al. (2008) measured elevated carotenoid level in *B. pendula* leaves sampled from pollutes area in comparison with samples from unpolluted one. They indicated that carotenoids perform many important physiological functions in plants: influence development and adaptation mechanisms, suggesting coordination of their synthesis in different physiological processes, but mostly serve as antioxidants against endogenous and exogenous oxidative stress. Oxidative stress caused by pollutants occurs when the amount of oxidants in the body or cell exceeds that of antioxidants. Only in Central area of Plovdiv we found in the autumn season an increment of carotenoids in leaf samples from all three deciduous trees. This area was characterized by intense motor traffic and was considered as heavy polluted. Simultaneously, only in West area we found an increment of chlorophyll/carotenoids ratio in the autumn, which site was regarded as less polluted from all selected. So, our findings, confirmed by the statistical evaluation, were in agreement with these authors. The normal ratio between chlorophyll and carotenoids content was 4.2-5 in sunny and 5.5-7 in shade leaves (Lichtenthaler, 1982). In our study these values not exceed 2.8 in all studied plant species, sampling areas and seasons, which probably was due to the impact of urban environment.

On the base of data obtained about nutrient and pigment content in the leaves, the health status of studied deciduous tree species could be defined as:

Acer platanoides – good in North, East and West areas, satisfactory in South-East and Central areas;

Aesculus hippocastanum - satisfactory in North, East and West areas, non-satisfactory in South-East and Central areas;

Betula pendula - satisfactory in Central and West areas, non-satisfactory in North, East and South-East areas.

We observed also serious damages in the development and duration of some phenophases for trees growing in more polluted areas. In September some visual symptoms were found: the necrosis and burn damages together with atypical coloring, premature leaf shedding, secondary leaf development and blossoming were more frequent in *B. pendula* and *A. hippocastanum* than in *A. platanoides* which crowns remained in full foliage until autumn. The results of the investigation on selected tree species revealed the main problems and unfavorable factors in the town of Plovdiv which affected their vigor and health status. The pollution sources may be combined into three major groups, which determine the character, degree and territorial range of negative impact: automobile transport, industrial sources and long distance transfer.

Conclusion

Urbanization causes complex relations between the anthropogenic factors and the natural environment. Thus, ecosystems with much altered soils, plant and animal communities are formed. The structure and functioning of these ecosystems is closely related to human activities. All areas of the green system of Plovdiv are subjected to a weaker or stronger degree of anthropogenic impact, which requires detailed research to determine the damages on particular species, their different tolerance capacity in anthropogenically influenced environment, their ability to serve as bioindicators of environmental pollution and as clearing filters. As green areas are one of the basic components of the urbanized territories, their construction and management should be based on precise data of the tolerance of the species to the changed ecological conditions this require complex studies for assessing the mechanisms of the interaction between the city condition and plants.

However, no baseline values exist to indicate which of these abnormalities is critical in individual cases. Since this kind of information may be of diagnostic assistance to maintain and management of urban green system, an approximation of critical concentrations need to be developed by establishing lowest and highest content of each nutrient at each period in the leaves of healthy green trees.

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